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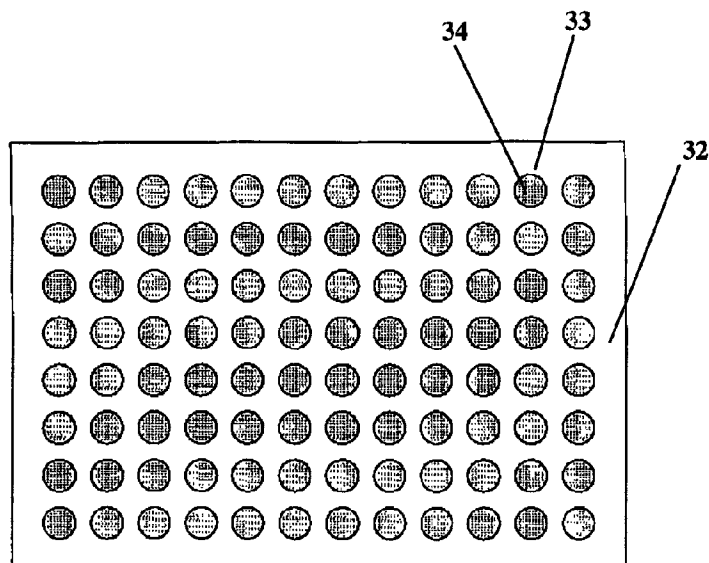
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(54) Title: POLYNUCLEOTIDE ANALYSIS USING COMBINATORIAL PCR

(57) Abstract: The invention comprises a two-step process for analysis of polynucleotides by chain extension of multiple oligonucleotide primers attached by 5'-linkages to solid supports by first performing PCR of the sample to be analysed in the presence of multiple oligonucleotides in solution, the nucleotide sequences of both sets of oligonucleotides being similar or identical. This produces immobilised single-stranded polynucleotides containing genetic sequence data derived from sample molecules. In a second step, support-bound polynucleotides are interrogated by hybridisation with a single labelled oligonucleotide probe or by second-strand synthesis with a primer-dependent polymerase using an oligonucleotide primer and nucleotide monomers, in which either or both of the primer and nucleotide monomers are labelled. Incorporation of label demonstrates the presence of two separate defined-sequence primers within the sample polynucleotide. The presence or absence within the sample of multiple combinations of primers are demonstrable in a single experiment by use of suitable apparatus, which may take the form of an oligonucleotide array. Multiple arrays may be accommodated in a 96-well format, allowing all possible combinations of 96 different primers to be generated simultaneously. If sequence data for the sample DNA is available in advance of the analysis, the results may be predicted by computer modelling, allowing design of highly-specific automated DNA-based assay procedures.

POLYNUCLEOTIDE ANALYSIS USING COMBINATORIAL PCR

This invention relates to methods and apparatus for analysis of biological materials. In particular the invention relates to methods and apparatus for the analysis of DNA and RNA

5   using the polymerase chain reaction (PCR).

The polymerase chain reaction is a technique which is well known to those skilled in the art of biochemical research, is widely used for isolation and analysis of biological samples and is described in many publications, for example "PCR" by C.R. Newton and A. Graham, published by Bios scientific publishers, Oxford 1997.

10   In general, the polymerase chain reaction makes use of two or more oligonucleotides which serve as primers for a primer-dependent DNA polymerase enzyme. This enzyme is used to produce multiple copies of double-stranded DNA molecules present in an analyte. By selection of suitable primers matching specific regions of the target DNA, selective amplification of those regions may be effected by repetitive cycling of the reaction mixture

15   through a temperature profile which includes the steps of denaturing of double-stranded DNA, annealing of oligonucleotide primers to the denaturated template DNA and primer extension using a thermostable DNA polymerase.

The use of oligonucleotides immobilised on solid supports as primers for PCR has been described in a number of documents. For example, US Patent No. 5,656,462 describes a

20   method of synthesising a polynucleotide immobilised support on PCR microplates, and World Patent No. WO 9932654 describes a method for performing RT-PCR on oligonucleotide-immobilised PCR microplates. In both these patent documents, the same oligonucleotide is immobilised on the surface of all the wells of the microplate in order to allow parallel processing of multiple samples of analytes.

25   US Patent No. 5,770,358 describes the use of PCR to amplify oligonucleotide "tags" bound to a solid-support bead in order to discriminate between different populations of such beads. US Patent No. 5,916,776 describes a method for amplification of a target nucleic acid in which copies of a first strand are captured on a solid support at a first location and moved to a second location at which copies of the first strand are regenerated. Procedures for

30   practising the method by use of magnetic particles and microfluidic devices are also disclosed. A further microfluidic device is described by the same applicants in US Patent 5,939,291.

The present invention provides processes and apparatus for analysis of DNA molecules by a combinatorial method which makes use of multiple oligonucleotide primers immobilised on solid supports.

5   Advantageously, the present invention provides a method and apparatus for analysis of DNA samples which requires no prior knowledge of the sequence information contained in the sample DNA, provides a large volume of data from a single experiment, and uses standard components and materials and is capable of mass-production.

10   According to the present invention, there is provided a process for transcription or amplification of sequence information from genetic material by chain extension of oligonucleotide primers characterised in that during the performance of the process:
       (i) at least two oligonucleotide primers are in solution, and
       (ii) at least two other oligonucleotide primers are attached to solid supports, and
15   (iii) the nucleotide-sequence of at least one solid-support-bound oligonucleotide is partially or completely identical with or complementary to the nucleotide-sequence of at least one oligonucleotide in solution.

The present invention further provides a process for transcription or amplification of
20   sequence information from a polynucleotide by chain extension of oligonucleotide primers characterised in that during the performance of the process:
       (i) at least two oligonucleotide primers are in solution, and
       (ii) at least one other oligonucleotide primer is attached to a solid support, and
       (iii) the nucleotide-sequence of at least one solid-support-bound oligonucleotide is partially
25   or completely identical with or complementary to part of the nucleotide-sequence of the polynucleotide.

The present invention further provides an apparatus for the analysis of analytes which comprises a multiplicity of solid support surfaces, a multiplicity of oligonucleotides or
30   polynucleotides attached to said solid support surfaces, such that each surface bears a single oligonucleotide or polynucleotide, identification means allowing each of said solid-support surfaces to be distinguished from other solid support surfaces, containment means allowing

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said solid-support surfaces to be contacted with solutions of analytes, enzymes or reagents, and closure means to allow agitation, heating or cooling without leakage.

The invention further provides an apparatus for the analysis of analytes which comprises a single solid support surface segregated into zones, a multiplicity of oligonucleotides or

5   polynucleotides attached to said zones, such that each zone bears a single oligonucleotide or polynucleotide, identification means allowing each of said zones to be distinguished from other zones, containment means allowing said solid-support surface to be contacted with solutions of analytes, enzymes or reagents, and closure means to allow agitation, heating or cooling without leakage.

10   The invention further provides an apparatus as hereinbefore defined further comprising containment means allowing any individual solid-support surface to be contacted with a specific analyte, enzyme or reagent without so contacting other solid-support surfaces, such that a different analyte, enzyme or reagent may be contacted with every individual solid-support surface.

15   The invention further provides apparatus as hereinbefore defined in which each solid support-surface or zone of said surface bears multiple oligonucleotides or polynucleotides in combination, such that each said surface or zone bears a different combination of oligonucleotides or polynucleotides, said combination comprising a sub-set of the totality of oligonucleotides or polynucleotides attached to all said surfaces or zones of the apparatus.

20

The invention further provides apparatus comprising a single solid support surface segregated into zones as hereinbefore defined further comprising division means allowing each of said zones to be physically separated from other zones, thereby enabling the entire support surface to be physically divided into individual zones.

25   The invention further provides a process for the preparation of multiple polynucleotides attached to a plurality of support surfaces which comprises the steps of:

(i) providing a plurality of support surfaces

(ii) covalently linking one or more oligonucleotides to each of said surfaces by means of a stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and

30   covalently linked to the support surface, such that each of said surfaces bears a different oligonucleotide or combination of oligonucleotides,

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(iii) contacting each of said support surfaces with one or more additional oligonucleotides and a nucleic acid or mixture of nucleic acids in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

5   (iv) optionally heating or altering the pH of said solid-supported oligonucleotide or oligonucleotides, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(v) optionally cooling or altering the pH of said solid-supported oligonucleotide or oligonucleotides, mixture of oligonucleotides and nucleic acids, enzyme or enzymes,

10   substrates and co-factors so as to effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface, and

(vi) optionally repeating steps (iv) to (v) one or more times in a cyclic manner, so as to effect conversion of individual oligonucleotides of the support surface to polynucleotides containing genetic sequence data derived from the nucleic acid or mixture of nucleic acids.

15

The invention further provides a process for the preparation of multiple polynucleotides attached to a support surface which comprises the steps of:

(i) providing a support surface

(ii) segregating the surface into discrete zones

20   (iii) covalently linking a plurality of oligonucleotides to said zones by means of a stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the support surface, such that each individual zone bears a different oligonucleotide or combination of oligonucleotides,

(iv) contacting said support surface with one or more additional oligonucleotides and a

25   nucleic acid or mixture of nucleic acids in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

(v) optionally heating or altering the pH of said solid-support-bound oligonucleotide or oligonucleotides, mixture of oligonucleotides and nucleic acids, enzyme or enzymes,

30   substrates and co-factors so as to effect dissociation of double-stranded molecules,

(vi) optionally cooling or altering the pH of said solid-support-bound oligonucleotide or oligonucleotides, mixture of oligonucleotides and nucleic acids, enzyme or enzymes,

substrates and co-factors so as to effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface, and (vii) optionally repeating steps (v) to (vi) one or more times in a cyclic manner, so as to effect conversion of individual oligonucleotides of the support surface to polynucleotides containing genetic sequence data derived from the nucleic acid or mixture of nucleic acids.

The invention further provides a process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

(i) applying the process as hereinbefore defined in order to produce a support surface or plurality of support surfaces bearing multiple polynucleotides containing genetic sequence data derived from the nucleic acid or mixture of nucleic acids,

(ii) contacting at least one of said support surfaces with one or more oligonucleotide or polynucleotide probes capable of hybridising to polynucleotides of the support surface or surfaces,

(iii) washing the support surface or surfaces to remove unhybridised oligonucleotide or polynucleotide probes,

(iv) measuring the quantity of oligonucleotide or polynucleotide probe hybridised to each polynucleotide of the support surface or surfaces, and

(v) optionally washing the support surface or surfaces under conditions sufficient to remove hybridised oligonucleotide or polynucleotide probes and repeating steps (ii) to (iv) one or more times using a different oligonucleotide or polynucleotide probe or probes in each iteration.

The invention further provides a process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

(i) applying the process as hereinbefore defined in order to produce a support surface or plurality of support surfaces bearing multiple polynucleotides containing genetic sequence data derived from the nucleic acid or mixture of nucleic acids,

(ii) contacting at least one of said support surfaces with one or more oligonucleotide primers in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to produce one or more polynucleotides complementary to the polynucleotides of the support surface or surfaces,

(iii) optionally heating or altering the pH of said support surface or surfaces, oligonucleotide primer or primers, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(iv) optionally cooling or altering the pH of said support surface or surfaces, oligonucleotide

5   primer or primers, enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides of the support surface or surfaces,

(v) optionally repeating steps (iii) to (iv) one or more times in a cyclic manner, so as to effect conversion of the primer oligonucleotide or oligonucleotides to polynucleotides complementary to the polynucleotides of the support surface or surfaces,

10   (vi) hybridising said complementary polynucleotides to the polynucleotides of the support surface or surfaces,

(vii) washing the support surface or surfaces to remove unhybridised polynucleotides,

(viii) measuring the quantity of complementary polynucleotide hybridised to each polynucleotide of the support surface or surfaces, and

15   (ix) optionally washing the support surface or surfaces under conditions sufficient to remove hybridised complementary polynucleotides and repeating steps (ii) to (viii) one or more times using a different oligonucleotide primer or primers in each iteration.

The invention further provides a process for the analysis of a nucleic acid or mixture of

20   nucleic acids which comprises the steps of:

(i) providing a multiplicity of support surfaces each equipped with unique identification means,

(ii) covalently linking each of a set of n oligonucleotides, where n is an integral number greater than unity, to one of said support surfaces by means of a stable linker moiety

25   covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the support surface, such that each individual oligonucleotide is attached to an individual support surface,

(iii) contacting all of said support-surfaces with a solution containing a mixture of all n oligonucleotides and the nucleic acid or mixture of nucleic acids to be analysed in the

30   presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides bound to the support surfaces,

(iv) optionally heating or altering the pH of said support surfaces, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(v) optionally cooling or altering the pH of said support surfaces, mixture of oligonucleotides

5   and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

(vi) optionally repeating steps (iv) and (v) one or more times in a cyclic manner, so as to produce n sets of support-bound polynucleotides by effecting conversion of individual

10   support-bound oligonucleotides to polynucleotides containing genetic sequence data derived from the mixtures of nucleic acids,

(vii) washing said support surfaces under denaturing conditions to denature double-stranded molecules and remove oligonucleotides or polynucleotides hybridised to said support-bound polynucleotides,

15   (viii) contacting one or more of said n support surfaces with a solution containing one of said n oligonucleotide primers in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to produce polynucleotides complementary to the polynucleotides bound to said support surfaces,

(ix) optionally heating or altering the pH of said support surfaces, oligonucleotide primers,

20   enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(x) optionally cooling or altering the pH of said support surfaces, oligonucleotide primers, enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides bound to said support surfaces,

25   (xi) optionally repeating steps (ix) to (x) one or more times in a cyclic manner, so as to effect conversion of the primer oligonucleotide to polynucleotides complementary to the polynucleotides bound to said support surfaces,

(xii) hybridising said complementary polynucleotides to the polynucleotides bound to said support surfaces,

30   (xiii) washing said support surfaces under non-denaturing conditions to remove unhybridised polynucleotides,

(xiv) separating each of said support surfaces from other support surfaces,

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(xv) washing each of said support surfaces under denaturing conditions to denature double-stranded molecules and release hybridised polynucleotides into solution,

(xvi) analysing the hybridised polynucleotides released into solution from each said support surface, and

5   (xvii) optionally repeating steps (viii) to (xvi) one or more times using a different member of the said set of n oligonucleotide primers in each iteration.

The invention further provides a process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

10   (i) providing a multiplicity of support surfaces each equipped with unique identification means,

(ii) covalently linking each of a set of n oligonucleotides, where n is an integral number greater than unity, to one of said support surfaces by means of a stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the

15   support surface, such that each individual oligonucleotide is attached to an individual support surface,

(iii) contacting all of said support-surfaces with a solution containing a mixture of all n oligonucleotides and the nucleic acid or mixture of nucleic acids to be analysed in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to

20   transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides bound to the support surfaces,

(iv) optionally heating or altering the pH of said support surfaces, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

25   (v) optionally cooling or altering the pH of said support surfaces, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

(vi) optionally repeating steps (iv) and (v) one or more times in a cyclic manner, so as to

30   produce n sets of support-bound polynucleotides by effecting conversion of individual support-bound oligonucleotides to polynucleotides containing genetic sequence data derived from the mixtures of nucleic acids,

(vii) washing said support surfaces under denaturing conditions to denature double-stranded molecules and remove oligonucleotides or polynucleotides hybridised to said support-bound polynucleotides,

(viii) individually contacting each of said n support surfaces with a solution containing one

5   of said n oligonucleotide primers in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to produce polynucleotides complementary to the polynucleotides bound to said support surface, such that every one of said n support surfaces is contacted with a unique member of the set of n oligonucleotide primers,

(ix) optionally heating or altering the pH of every said support surface, oligonucleotide

10   primer, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(x) optionally cooling or altering the pH of every said support surface, oligonucleotide primer, enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides bound to said support surface,

15   (xi) optionally repeating steps (ix) to (x) one or more times in a cyclic manner, so as to effect conversion of the primer oligonucleotide to polynucleotides complementary to the polynucleotides bound to said support surface,

(xii) hybridising said complementary polynucleotides to the polynucleotides bound to said support surface,

20   (xiii) washing said support surface under non-denaturing conditions to remove unhybridised polynucleotides,

(xiv) washing said support surface under denaturing conditions to denature double-stranded molecules and release hybridised polynucleotides into solution,

(xv) analysing the hybridised polynucleotides released into solution from each said support

25   surface, and

(xvi) optionally repeating steps (viii) to (xv) one or more times using for each of said n support surfaces a different member of the said set of n oligonucleotide primers in each iteration.

30   The invention further provides a process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

(i) providing a support surface,

(ii) segregating the surface into n discrete zones, where n is an integral number greater than unity,

(iii) providing each of said zones with unique identification means,

(iv) covalently linking each of a set of n oligonucleotides to one of said zones by means of a stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the support surface, such that each individual oligonucleotide is attached to an individual zone of the support surface,

(v) contacting said support-surface with a solution containing a mixture of all n oligonucleotides and the nucleic acid or mixture of nucleic acids to be analysed in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides attached to the support surface,

(vi) optionally heating or altering the pH of said support surface, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(vii) optionally cooling or altering the pH of said support surface, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

(viii) optionally repeating steps (vi) and (vii) one or more times in a cyclic manner, so as to produce n sets of support-bound polynucleotides by effecting conversion of individual support-bound oligonucleotides to polynucleotides containing genetic sequence data derived from the mixtures of nucleic acids,

(ix) washing said support zones under denaturing conditions to denature double-stranded molecules and remove oligonucleotides or polynucleotides hybridised to said support-bound polynucleotides,

(x) contacting one or more of said n support zones with a solution containing one of said n oligonucleotide primers in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to produce polynucleotides complementary to the polynucleotides bound to said support zones,

(xi) optionally heating or altering the pH of said support zones, oligonucleotide primer,
enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded
molecules,

(xii) optionally cooling or altering the pH of said support zones, oligonucleotide primer,
enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides
complementary to the polynucleotides bound to said support zones,

(xiii) optionally repeating steps (xi) to (xii) one or more times in a cyclic manner, so as to
effect conversion of the primer oligonucleotide to polynucleotides complementary to the
polynucleotides bound to said support zones,

(xiv) hybridising said complementary polynucleotides to the polynucleotides bound to said
support zones,

(xv) washing said support zones under non-denaturing conditions to remove unhybridised
polynucleotides,

(xvi) separating each of said support zones from other support zones,

(xvii) washing each said support zone under denaturing conditions to denature double-
stranded molecules and release hybridised polynucleotides into solution,

(xviii) analysing the hybridised polynucleotides released into solution from each said support
zone, and

(xix) optionally repeating steps (x) to (xviii) one or more times using in each iteration a
solution of a different oligonucleotide primer selected from the set of n oligonucleotide
primers.

The invention further provides a process for the analysis of a nucleic acid or mixture of
nucleic acids which comprises the steps of:

(i) providing a support surface,

(ii) segregating the surface into n discrete zones, where n is an integral number greater than
unity,

(iii) providing each of said zones with unique identification means,

(iv) covalently linking each of a set of n oligonucleotides to one of said zones by means of a
stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and
covalently linked to the support surface, such that each individual oligonucleotide is attached
to an individual zone of the support surface,

(v) contacting said support-surface with a solution containing a mixture of all n
oligonucleotides and the nucleic acid or mixture of nucleic acids to be analysed in the
presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to
transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the

5   oligonucleotides attached to the support surface,

(vi) optionally heating or altering the pH of said support surface, mixture of oligonucleotides
and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation
of double-stranded molecules,

(vii) optionally cooling or altering the pH of said support surface, mixture of

10   oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to
effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic
acids to the oligonucleotides of the support surface,

(viii) optionally repeating steps (vi) and (vii) one or more times in a cyclic manner, so as to
produce n sets of support-bound polynucleotides by effecting conversion of individual

15   support-bound oligonucleotides to polynucleotides containing genetic sequence data derived
from the mixtures of nucleic acids,

(ix) washing said support surface under denaturing conditions to denature double-stranded
molecules and remove oligonucleotides or polynucleotides hybridised to said support-bound
polynucleotides,

20   (x) physically dividing the support surface to form n individually-identifiable support zones
each bearing a set of support-bound polynucleotides derived by extension of one of the n
support-bound oligonucleotide primers,

(xi) individually contacting every one of said n support zones with a solution containing one
of said n oligonucleotide primers in the presence of an enzyme or enzymes together with

25   enzyme substrates and co-factors so as to produce polynucleotides complementary to the
polynucleotides bound to said support zone, such that every one of said n support zones is
contacted with a unique member of the set of n oligonucleotide primers,

(xii) optionally heating or altering the pH of every said support zone, oligonucleotide primer,
enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded

30   molecules,

(xiii) optionally cooling or altering the pH of every said support zone, oligonucleotide primer, enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides bound to said support zone,

(xiv) optionally repeating steps (xii) to (xiii) one or more times in a cyclic manner, so as to

5   effect conversion of the primer oligonucleotide to polynucleotides complementary to the polynucleotides bound to said support zone,

(xv) hybridising said complementary polynucleotides to the polynucleotides bound to said support zone,

(xvi) washing said support zone under non-denaturing conditions to remove unhybridised

10   polynucleotides,

(xvii) washing said support zone under denaturing conditions to denature double-stranded molecules and release hybridised polynucleotides into solution,

(xviii) analysing the hybridised polynucleotides released into solution from each said support zone, and

15   (xix) optionally repeating steps (xi) to (xviii) one or more times using for each of said n support zones a different member of the said set of n oligonucleotide primers in each iteration.

The invention further provides a process for the analysis of two or more distinct mixtures of

20   nucleic acids which comprises the steps of:

(i) applying the processes as hereinbefore defined in order to produce a set of support surfaces or zones bearing a first set of immobilised polynucleotides containing genetic sequence data derived from a first mixture of nucleic acids, together with a first set of polynucleotides complementary to said first set of immobilised polynucleotides,

25   (ii) applying the processes as hereinbefore defined in order to produce a set of support surfaces or zones bearing a second set of immobilised polynucleotides containing genetic sequence data derived from a second mixture of nucleic acids, together with a second set of polynucleotides complementary to said second set of immobilised polynucleotides,

(iii) hybridising said first set of complementary polynucleotides to said first set of

30   immobilised polynucleotides,

(iv) individually releasing and analysing the complementary polynucleotides hybridised to each of said first set of immobilised polynucleotides,

(v) hybridising said second set of complementary polynucleotides to said second set of immobilised polynucleotides

(vi) individually releasing and analysing the complementary polynucleotides hybridised to each of said second set of immobilised polynucleotides,

5   (vii) optionally hybridising one or more members of said first set of complementary polynucleotides to one or more members of said second set of immobilised polynucleotides, individually releasing and analysing the complementary polynucleotides hybridised to each of said second set of immobilised polynucleotides,

(viii) optionally hybridising one or more members of said second set of complementary

10   polynucleotides to one or more members of said first set of immobilised polynucleotides, individually releasing and analysing the complementary polynucleotides hybridised to each of said first set of immobilised polynucleotides, and

(ix) optionally repeating steps (ii) and (v) to (viii) for further mixtures of nucleic acids.

15   The invention further provides a process as hereinbefore defined in which a single support surface segregated into zones is physically divided into discrete zones after completing at least one step of the process and each such zone is processed separately during subsequent steps of the process.

20   The invention further provides a process as hereinbefore defined in which the stable linker moiety is covalently linked to the 5'-terminus of each oligonucleotide by means of covalent bonding to one or more atoms of the carbohydrate moiety of the 5'-terminal nucleotide.

The invention further provides a process as hereinbefore defined in which the stable linker

25   moiety is covalently linked to the 5'-terminus of each oligonucleotide by means of covalent bonding to one or more atoms of the heterocyclic moiety of the 5'-terminal nucleotide.

The invention further provides a process as hereinbefore defined in which the stable linker moiety comprises a covalently-bound functionality with a chemical equivalence of between 100 and 5000 picomoles per square millimetre of support surface.

30   The invention further provides a process as hereinbefore defined in which the stable linker moiety is thermally stable in aqueous solution at 95 deg. C.

The invention further provides a process as hereinbefore defined in which the stable linker moiety is hydrolytically stable in aqueous solution between pH 4 and pH 11.

The invention further provides a process as hereinbefore defined in which the oligonucleotides contain internucleotide linkages selected from the group comprising

5   phosphodiesters, phosphorothioates and methyl phosphonates.

The invention further provides a process as hereinbefore defined in which one or more oligonucleotides contain labelled molecules selected from the group comprising fluorescent molecules, luminescent molecules, biotin-labelled molecules, radiolabelled molecules and enzyme conjugates.

10 The invention further provides a process as hereinbefore defined in which the nucleic acid or mixture of nucleic acids to be analysed is selected from the group comprising single-stranded DNA molecules, double-stranded DNA molecules and messenger RNA molecules.

The invention further provides a process as hereinbefore defined in which the enzyme or

15 enzymes are selected from the group comprising DNA polymerases, thermostable DNA polymerases, reverse transcriptases, thermostable reverse transcriptases, DNA ligases and thermostable DNA ligases.

The invention further provides a process as hereinbefore defined in which the enzyme substrates and co-factors are selected from the group comprising nucleoside triphosphates,

20 fluorescent nucleoside triphosphates, luminescent nucleoside triphosphates, biotin-labelled nucleoside triphosphates, amino-allyl nucleoside triphosphates, radiolabelled nucleoside triphosphates, metal salts, buffering agents, chelating agents, chaotropic agents and nuclease inhibitors.

The invention further provides an apparatus as hereinbefore defined in which the support

25 surface, containment means and closure means comprise separate components adapted to be assembled to form the apparatus.

The invention further provides an apparatus as hereinbefore defined in which each set of immobilised oligonucleotides or polynucleotides further comprises control substances or indicia adapted for identification, alignment or calibration purposes.

30 The invention further provides apparatus and processes as hereinbefore defined further comprising automated handling equipment allowing high-throughput analysis of DNA samples under computer control.

The invention further provides a computer program comprising software code portions for controlling apparatus adapted to perform the process of the invention as hereinbefore defined.

5   The invention further provides a computer program comprising software code portions for predicting the outcome of the process of the invention as hereinbefore defined for any given combination of oligonucleotides in relation to the presence or absence in an analyte of one or more polynucleotides.

10   Specific embodiments of the invention will now be described, by way of example, with reference to the accompanying drawings, in which:-

Figure 1 shows a radio-frequency transponder sealed in a glass envelope whose surface may be used as a solid support surface in the practice of the invention.

15   Figure 2 shows a glass plate divided into zones which may be used as a solid support surface in the practice of the invention.

Figure 3 shows a silicon wafer divided into zones which may be used as a solid support surface in the practice of the invention.

Figure 4 shows schematically a process for performing PCR on multiple solid supports.

20   Figure 5 shows schematically a process for generating labelled polynucleotides which are complementary to immobilised polynucleotides produced by PCR.

Figure 6 shows schematically procedures for analysis of complementary polynucleotides.

Figure 7 shows schematically a process for performing PCR on a single solid support subdivided into zones.

25   Figure 8 shows schematically a process for generating labelled complementary sequences after separating a single solid support into individual zones.

Figure 9 shows, in plan view, a 96-well plate in which each well contains an oligonucleotide array.

Figure 10 shows, in plan view, an individual oligonucleotide array.

30   Figure 11 shows, in exploded view, the components of a modular 96-well plate.

Figures 12 to 15 show, in sectional view, the assembly of the components of a modular 96-well plate mounted between heating blocks.

Figure 16 shows, in sectional view, the detailed construction of an individual well of a 96-well plate.

Figure 17 shows a table of oligonucleotide sequences which may be used in the practice of the invention.

5   Figure 18 shows schematically a process for distinguishing between two DNA samples by practising the process of the invention.

In a preferred embodiment of the apparatus of the invention, the support surface is the external glass surface of a radio-frequency tag (RF tag) as illustrated in Figure 1. Referring to

10   the drawing, this consists of a microelectronic radio-frequency transponder circuit 1 equipped with an antenna coil 2 wound on a ferrite core 3. The entire assembly is hermetically sealed in a glass envelope 4 whose outer surface 5 serves as the support surface for immobilised oligonucleotides in the practice of the invention.

In a second preferred embodiment of the apparatus of the invention, the support surface is

15   the surface of a glass plate divided into zones as illustrated in Figure 2. Referring to the drawing, the surface of the glass plate is divided into zones by means of a grid of lines 6 which are scribed into the surface, for example by use of a glass knife or a diamond pencil, or etched into the surface by chemical treatment, for example with hydrofluoric acid, or by laser ablation. Zones are also equipped with unique identifying marks 7.

20   In a third preferred embodiment of the apparatus of the invention, the support surface is the surface of a silicon wafer divided into zones as illustrated in Figure 3. Referring to the drawing, the surface of the silicon wafer is divided into zones by means of a grid of lines 8 which are formed in the surface, for example by laser ablation. Zones are also equipped with unique identifying marks 9 in the form of a two-dimensional bar code formed by laser

25   ablation. Prior to use of the wafer as a solid-support for oligonucleotide immobilisation, a layer of silicon oxide is formed on the surface of the wafer using standard microelectronic fabrication methods.

A preferred method of practising the invention is illustrated schematically in Figure 4. According to this method, a set 10 of oligonucleotide primers in solution is used in

30   conjunction with a set 11 of the same oligonucleotide primers each of which is attached to one of a set of individual support surfaces 12 to 19 inclusive. Each of the solid support surfaces may be a discrete entity, for example an RF tag as hereinbefore described and

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illustrated in Figure 1, or may be a zone of a larger surface, for example the glass plate of Figure 2 or the silicon wafer of Figure 3. Other types of support surfaces suitable for the practice of the invention include glass or plastic beads labelled with uniquely-identifiable markers, such as defined-ratio combinations of fluorescent dyes or specific combinations of

5   other molecules. Support media of these types are commercially available for applications in combinatorial chemistry and biochemical analysis.

In a first step of the method of this embodiment, the set 11 of oligonucleotide primers attached to the solid-support surfaces 12 to 19 inclusive is contacted with a solution containing a set 10 of the same oligonucleotide primers together with the sample 20 of

10   double-stranded DNA molecules to be analysed, a polymerase enzyme 21, for example *Taq* DNA polymerase, a mixture 22 of unlabelled deoxynucleoside triphosphates comprising A- ,G-,C- and T- deoxynucleoside triphosphates, and a mixture 23 of magnesium chloride and buffer salts, the solution being loaded into a sealable container which is adapted to contain all of the solid-support surfaces 12 to 19 inclusive. A wide variety of containers are suitable

15   for the practice of the invention, for example microcentrifuge tubes may be used where RF tags are employed as solid-supports, or alternatively a sealable multi-well plate is also suitable. Where a single support surface subdivided into zones is used in the practice of the invention, sealable containers such as those employed for staining microscope slides may be employed. After physical division of such support surfaces, the individual zones may be

20   transferred to microcentrifuge tubes for subsequent steps of the method of the invention. Apparatus designed for combinatorial chemical synthesis may also be employed in practising the method of the invention. For example, reactor blocks containing multiple reaction chambers are suitable for use with RF tags or with individual zones of support surfaces after physical division of a single support surface subdivided into zones.

25   As will be understood by those skilled in the art of biochemical analysis, a wide variety of containers other than those mentioned here may also be employed in practising the method without departing from the spirit of the invention.

The sealed container is then loaded into a heating block or thermal cycler suitable for performing the Polymerase Chain Reaction (PCR) and subjected to multiple amplification

30   cycles each comprising a denaturation step, an annealing step and an extension step. During each amplification cycle, segments of double-stranded DNA sequences in the sample to be analysed are amplified by PCR, the section of each such sequence amplified being

determined by the presence and location of segments within each sequence possessing complementarity to one or more pairs of oligonucleotide primers.

After multiple cycles of amplification, the solution contains amplified copies of DNA segments contained within the sample to be analysed, formed by extension of members of

5 the set 10 of oligonucleotide primers originally present in solution. In addition, the set 11 of solid-support-bound oligonucleotide primers also participates in the amplification process, so that each support surface also bears covalently-bound amplified copies of DNA segments contained within the sample to be analysed.

After completion of the PCR process, the solution is removed and the support surfaces are

10 washed under denaturing conditions in order to remove all non-covalently-linked oligonucleotides and polynucleotides. After this operation, the set of solid-support surfaces carries a set 24 of single-stranded polynucleotides derived by amplification of molecules of the sample DNA 20 and covalently bound to the solid supports.

In a second step of the method of this embodiment, as illustrated schematically in Figure 5,

15 the set 24 of solid-support-bound single-stranded polynucleotides is subjected to a further primer-dependent polymerase reaction in which the sealable container accommodating the solid-supports is loaded with a solution containing a single oligonucleotide primer 25 which may be selected from the set 10 of oligonucleotide primers employed in the first step of the method. Alternatively, an unrelated oligonucleotide primer may be used. This

20 oligonucleotide acts as a primer for synthesis of a complementary strand on each of the set 24 of solid-support-bound single-stranded polynucleotides. The solution also contains a polymerase enzyme 26, for example *Taq* DNA polymerase, together with magnesium chloride and buffer salts, and a mixture 27 of deoxy nucleoside triphosphates, comprising A- ,G-,C- and T- deoxynucleoside triphosphates, together with a labelled molecule. The labelled

25 molecule is one which may be incorporated into the complementary strand formed on each polynucleotide during the primer-dependent polymerase reaction. For example, a Uridine derivative such as biotin-16-dUTP or aminoallyl-dUTP may be included in the polymerase reaction mixture in order to incorporate biotin labelled molecules or aminoallyl substituents suitable for labelling in place of Thymidine units within the complementary strand during the

30 primer-dependent polymerase reaction. Alternatively, the label may be attached to the 5'- terminus of the oligonucleotide primer, as is commonly employed in DNA sequencing. In this case fluorescent dyes such as those commonly known as FAM, JOE, TAMRA and ROX,

which are commercially available from Applied Biosystems, may be incorporated into the oligonucleotide primer during synthesis. Similarly, a 5'-biotin label may be incorporated into the oligonucleotide during synthesis by use of a biotin phosphoramidite. Radioactive labels may also be used where appropriate. Where the label is attached to the oligonucleotide, the

5   subsequent polymerase-mediated reaction may be omitted and replaced by a simple hybridisation process in which the labelled oligonucleotide serves as a probe for detection of complementary sequences contained in the solid-support-bound single-stranded polynucleotides. However, greater sensitivity may be obtained by incorporating multiple labels into the second strand by performing the polymerase reaction in the presence of

10   labelled nucleotide triphosphates. Alternatively, different labels may be incorporated into the oligonucleotide primer and the nucleotide triphosphates, allowing determination of the ratio of 5'-terminal label to labels incorporated during second-strand synthesis. This may be used, for example, to obtain estimates of the chain length and base composition of the second strand. Where suitable detection means are available, multiple fluorescent dyes or other

15   labels may be used for this purpose, such that each of the four nucleotide triphosphates contains a different dye, allowing the labels associated with each nucleotide to be determined separately. Where appropriate, multiple sets of reactions may be performed using the same combinations of oligonucleotides but with different labelled nucleotides in each reaction, providing further levels of nucleotide-specific information.

20   The primer-dependent polymerase reaction may be driven to completion by subjecting the solution to thermal cycling as described in the foregoing description of the first step of the method of this embodiment. In this second step, however, the polymerisation products are formed in solution rather than on the solid-support surface, the set 24 of solid-support-bound single-stranded polynucleotides serving as the templates for second-strand synthesis. The

25   resulting solution contains labelled polynucleotides complementary to the solid-support-bound polynucleotides which are then allowed to hybridise together to form double-stranded polynucleotides in which one strand is bound to the solid-support surface. After washing to remove unbound material, the quantity of label attached to each support surface is then determined using standard procedures for quantitation of fluorescent or luminescent

30   molecules or, in the case of a radioactive label, radiometry or autoradiography. Where appropriate, support surfaces divided into zones such as those illustrated in Figure 2 and Figure 3 may first be physically divided before quantitation of the labelled molecules

attached to each zone. The divided zones may then be quantitated individually, for example by elution of the labelled molecule prior to spectrophotometric, spectrofluorimetric or radiometric determination of the eluate. The process of elution and quantitation is illustrated schematically in Figure 6, in which a double-stranded polynucleotide 29 attached to an

5   individual support zone is heated in an appropriate buffer solution in order to dissociate the polynucleotide into a single-stranded support-bound polynucleotide 30 and the labelled complementary strand 31, which is released into solution. After removal of the solid support, the concentration in solution of the labelled molecules is determined by spectrometry, spectrofluorimetry or radiometry as appropriate.

10   As will be apparent to those skilled in the art of DNA sequence analysis, the method of the invention as described above may be modified in a variety of ways without departing from the spirit of the invention. For example, the set of solid-support-bound polynucleotides produced in the first step of the method may be applied directly in other techniques for which such supported molecules are employed, as in the preparation and application of DNA

15   micro-arrays for detection of genetic sequences by hybridisation. Similarly, the amplification of genetic sequence information from sample molecules by PCR may be performed in two stages, using only the solution-phase primers initially in order to obtain a preliminary degree of amplification before introducing the solid-supported primers. Where appropriate, a purification or concentration step may be included after such initial amplification in order to

20   remove residual sample DNA or selectively carry forward specific amplification products for incorporation into the solid-support-bound polynucleotides by further cycles of PCR. Similarly, other detection strategies may be adopted in order to eliminate the requirement for labelling, for example by using mass-spectrometric or electrochemical techniques to characterise the polynucleotides produced.

25

The method of the invention as applied to a single support surface divided into support zones is further illustrated schematically in Figure 7, which summarises the steps involved in production of single-stranded PCR products attached to the solid-support zones and in Figure 8, which summarises the subsequent operations of complementary strand synthesis,

30   separation of the support into individual zones, and elution and quantitation of labelled complementary strands.

Alternatively, each zone of the surface may be quantitated individually without physical division of the support surface where an appropriate instrumental technique for doing so is available, for example by use of a laser scanner where fluorescently-labelled molecules are to be determined. Individual support surfaces are identified by reading the identification means

5   from each surface, whether these be attached or engraved indicia as in the surface zones of Figure 2 and Figure 3 or coded RF tags as in Figure 1.

Since the sequence of the 5'-terminus of each solid-support-bound polynucleotide is known from the sequence of the oligonucleotide initially applied to that support surface, and the sequence of the 3'-terminus of each solid-support-bound polynucleotide is known to be

10   complementary to the single primer oligonucleotide present in solution in the second step of the method, the results from all support surfaces may be used to deduce sequence information for the sample to be analysed. For example, the sequences of the primer oligonucleotides may be selected so as to demonstrate the presence or absence of a specific polynucleotide in the analyte which is associated with a microbial infection or other disease

15   condition or of a genetic disorder.

The second step of the method may be repeated using the same set of support-bound polynucleotides with a different primer oligonucleotide in solution in order to obtain further sequence information. This procedure may be applied iteratively so as to examine all

20   possible combinations of the chosen set of primer oligonucleotides. In the example illustrated schematically in Figure 4, where a set of eight primer oligonucleotides is employed, there are 64 possible combinations of the primer oligonucleotides which may be examined in this manner.

A particular advantage of the process of this embodiment is that it has the capacity to provide

25   verification of results, since, if double-stranded template DNA is being analysed, each of the possible pairs of oligonucleotides should produce two signals, one for the combination of a solid-support-bound primer P_1 with a solution-phase primer P_2, and a corresponding signal for the complementary strand represented by the combination of solid-support-bound primer P_2 with solution-phase primer P_1.

30   As will be appreciated by those skilled in the art of DNA analysis and combinatorial chemistry, much larger sets of primer oligonucleotides than those depicted in this figurative

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example may be employed in order to generate results from hundreds or thousands of combinations of the primer oligonucleotides.

A fourth preferred embodiment of the apparatus of the invention comprises an apparatus for

5   analysis of DNA samples using a set of 96 primer oligonucleotides, as illustrated in Figure 9. In this embodiment, the support surface is formed into wells, the base of each well being planar and bearing an array of oligonucleotides. Referring to the drawings, Figure 9 shows in plan view a 96-well plate 32 in which each well 33 contains an array 34 of oligonucleotides. A detailed plan view of an individual array is shown in Figure 10, in which the circular base

10   35 carries multiple sub-zones 36 to each of which is attached an oligonucleotide. Each oligonucleotide is attached to the surface by means of a stable linker moiety covalently linked to the 5'-terminus of the oligonucleotide and covalently linked to the support surface. The stable linker moiety should be thermally stable in aqueous solution at 95 deg.C and hydrolytically stable between pH 4 and pH 11, allowing the linkage of oligonucleotide to the

15   surface to withstand the conditions normally employed in practising techniques such as the polymerase chain reaction (PCR). The linker moiety may incorporate spacer molecules as part of the covalent linkage between oligonucleotide and support surface, in order to reduce or eliminate steric effects which may inhibit processes involving the oligonucleotides, for example binding of oligonucleotides to an enzyme molecule.

20   The identity of the oligonucleotide attached to each position of the array is established at the time of manufacture, so that individual identification means for each oligonucleotide are not required, the position of the oligonucleotide within the array serving to identify it uniquely. Additional positions may be included in each array to provide loci for placement of control substances and index markers for calibration and alignment purposes, especially where

25   automated systems are to be used in quantitation and data acquisition. This is exemplified in Figure 10, which contains eight additional sub-zones 35 in addition to the ninety-six zones required for placement of oligonucleotides.

The attachment of oligonucleotides to the planar base of each well may be performed directly on the material of the 96-well plate, for example by attaching suitable linker molecules to a

30   polystyrene or polypropylene surface as described, for example, in the above-referenced patents US 5,656,462 and WO 9932654. For ease of manufacture, however, it is preferable to attach the oligonucleotides to a planar slice of support material which may then be inserted

into the base of each well. This permits the mass-production of the body of the 96-well plate

from plastic materials suitable for use in an injection-moulding process while allowing other

materials to be used as the support surface for the array which may then be fixed in position

by an interference fit or by welding or using a suitable adhesive. Preferred materials for the

5   planar slice are glass or a silicon oxide layer formed on a silicon wafer, and the slice may be

of any suitable shape, for example circular, square, rectangular or hexagonal in form.

In a fifth preferred embodiment of the apparatus of the invention, a 96-well plate of modular

construction is provided. This has three major components: a cover plate, a body divided into

10   96 cylindrical compartments opening on to both faces of the body, and a baseplate carrying

96 oligonucleotide arrays. These components may be combined to form a stack as illustrated

in Figure 11, in which the cover plate 37 overlies the body section 38, which in turn is placed

over the baseplate 39 carrying the arrays 40. The assembly of the stack is shown in sectional

view in Figure 12, in which the cover plate 37 overlies the body section 38, which in turn is

15   placed over the baseplate 39 carrying the arrays 40. O-ring seals 45 and 46 are fitted to the

upper and lower ends of each of the cylindrical bores in the body component to form a fluid-

tight seal against the cover plate and the baseplate.

In use, as illustrated in Figure 13, the baseplate and body are fitted to a metal base 41 and

20   held in place by spring clips 42 and 43. At this stage, each well is sealed at its lower end

against the baseplate and may be charged with solutions appropriate to the analysis to be

performed. Following this, as illustrated in Figure 14, the cover plate 37 may be placed in

position and held in place by a metal pressure plate 44. This serves to seal the upper end of

each well against the cover plate. As illustrated in Figure 15, where thermal cycling is

25   required, for example in order to perform polymerase chain reactions on the arrays, the entire

assembly may now be compressed between two heated metal plates 47 and 48, the upper

plate serving to prevent condensation on the underside of the cover plate. Where "hot start"

conditions are required, the metal base 41 may be placed on a heated surface prior to loading

solutions in the wells, following which the cover plate 37 and pressure plate 44 may be

30   fitted.

The internal arrangement of an individual well of the assembled apparatus is illustrated in

Figure 16, in which the baseplate 39 bearing the oligonucleotide array 40 is supported on

metal base 41, the cylindrical bore of the body component 38 is sealed against the baseplate by O-ring seal 46 and by O-ring seal 45 against the cover plate 37 which is held in place by pressure plate 44.

5   Where, as hereinbefore described, the process of the invention is to be performed using an initial amplification step using only solution-phase primers before introducing the solid-support bound primers, this may be accomplished simply by initially inverting the apparatus during the first step of the process, so that the baseplate 39 bearing the oligonucleotide arrays is uppermost. In this configuration, the solution in each well is not in contact with the solid-

10   support bound oligonucleotides of the array surfaces, and PCR proceeds using only solution-phase primers. After the initial amplification step, the apparatus is inverted again, allowing the solid-support bound primers to participate in subsequent cycles of PCR amplification. Preferred materials for the construction of the metal base 41 and pressure plate 44 are aluminium or stainless steel. Preferred materials for the construction of the body 38 and

15   cover plate 37 are polypropylene, polytetrafluorethylene (PTFE), polyether ether ketone (PEEK) or polystyrene. A preferred material for the construction of the baseplate 39 is glass. Preferred materials for the O-ring seals are fluorocarbon rubber or EPDM.

The arrays 40 may be formed directly on the surface of the baseplate 39 using a commercial

20   robotic arraying system. Alternatively, the arrays may be manufactured individually on plates of glass or surface-oxidised silicon wafers as hereinbefore described and fixed to the baseplate 39 by welding or by using a suitable adhesive, in which case the baseplate may be manufactured from a plastic material such as polypropylene or polystyrene. The plates carrying the arrays may be disc-shaped, as depicted in Figure 10, or may equally be

25   rectangular, square or hexagonal in form. Where silicon wafers are used for construction of arrays, a square or rectangular form may be preferred to facilitate handling of diced wafer sections by use of standard microfabrication and handling equipment.

This embodiment of the apparatus of the invention allows all possible combinations of 96

30   oligonucleotides to be generated in a single analysis. By analysing the results from multiple experiments, information may be deduced as to the sequence of DNA molecules of the sample, particularly by using appropriate computer programs to interpret the data generated.

The 96-well layout may be further extended to encompass 384-well or 1536-well formats, enabling many more combinations of primers to be generated.

A particularly useful aspect of the procedures of the invention is the ability to discriminate

5   between populations of DNA molecules even where little is known about the genetic sequences involved. This can be achieved by choosing primers from sets of oligonucleotides which are selected randomly, with the proviso that their (G+C) content should be between 60% and 70% and that they should not have self-complementary ends. These criteria ensure that the oligonucleotides will prime efficiently during the PCR process. Such random sets of

10   oligonucleotides are commonly used in the technique of Random Amplified Polymorphic DNA (RAPD) production by PCR, and are commercially available. Examples of typical 10-base primer sets are given in Figure 17. The nucleotide sequences given in this diagram are for illustrative purposes only and do not represent the sequences of actual biological materials.

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In a second preferred embodiment of the process of the invention, individual support surfaces are functionalised with a mixture of two or more oligonucleotides in combination prior to practising the method hereinbefore described. This allows results to be obtained for one or more sub-sets of the set of oligonucleotides. This may be useful, for example, as a

20   preliminary screening method prior to refining the method in subsequent experiments by using individual support-bound oligonucleotides as in the hereinbefore-described method.

In a third preferred embodiment of the process of the invention, the solution used during the second step of the method, in which complementary strand synthesis is performed, contains a

25   mixture of two or more oligonucleotides in combination. The method is otherwise practised as hereinbefore described. This allows results to be obtained for one or more sub-sets of the set of oligonucleotides. This may be useful, for example, as a preliminary screening method prior to refining the method in subsequent experiments by using individual support-bound oligonucleotides as in the hereinbefore-described method.

30

In a fourth preferred embodiment of the process of the invention, individual support surfaces are functionalised with a mixture of two or more oligonucleotides in combination and the

solution used during the second step of the method, in which complementary strand synthesis is performed, contains a mixture of two or more oligonucleotides in combination. The method is otherwise practised as hereinbefore described. This allows results to be obtained for one or more sub-sets of the set of oligonucleotides. This may be useful, for example, as a

5   preliminary screening method prior to refining the method in subsequent experiments by using individual support-bound oligonucleotides as in the hereinbefore-described method.

In a fifth preferred embodiment of the process of the invention, solutions of two or more different oligonucleotides are applied separately to individual solid-supports or solid-support

10   zones during the second step of the method, in which complementary strand synthesis is performed. The method is otherwise practised as hereinbefore described. This requires separate containment means for the individual solid-supports or solid-support zones. Where the individual solid-supports are separate entities, for example RF tags as illustrated in figure 1, or a single support surface which has been physically divided into individual support

15   zones as hereinbefore described, this may be accomplished by placing each support entity in a separate tube or other container prior to performing the second step of the method as hereinbefore described. In the case of a single support surface subdivided into zones which have not been physically divided, this may be accomplished by applying a suitable gasket to the support surface to allow different solutions to be applied to each zone without cross-

20   contamination.

In a sixth preferred embodiment of the process of the invention, solutions of two or more different DNA samples are applied separately to individual solid-supports or solid-support zones during the first step of the method, in which PCR amplification products are formed

25   on the solid-supports. The method is otherwise practised as hereinbefore described. This requires separate containment means for the individual solid-supports or solid-support zones. Where the individual solid-supports are separate entities, for example RF tags as illustrated in figure 1, this may be accomplished by placing each support in a separate tube or other container prior to performing the method as hereinbefore described. In the case of a single

30   support surface subdivided into zones, this may be accomplished by applying a suitable gasket to the support surface to allow different solutions to be applied to each zone without cross-contamination.

In a seventh preferred embodiment of the process of the invention, solutions of labelled complementary strands eluted from one support surface are applied to another support surface and allowed to hybridise thereto. Following this operation, the support surface is

5 washed to remove unbound material and hybridised polynucleotide is eluted by washing under more stringent conditions and quantitated by the appropriate method. This procedure may be repeated for all possible combinations of such eluted complementary strands and support surfaces and the data thereby obtained applied in various ways, for example in the construction of a linkage map for the DNA sample of the analyte.

10

As will be apparent to those skilled in the arts of biochemical analysis and DNA technology, a large number of different combinations of oligonucleotides may be selected from the total number of possible oligonucleotides of any given chain-length, and each combination will give different results for any given sample of DNA which may be subjected to analysis by

15 practising the invention. Very large volumes of data may therefore be generated by performing multiple analyses of a sample using different combinations of oligonucleotides in each case. As will also be apparent to those skilled in the art of biochemical analysis, the statistical probability of finding a match between any given oligonucleotide and a sub-sequence of a component DNA molecule within a given sample is a function of the length of

20 the oligonucleotide, and this parameter may be varied as required in order to obtain informative results from the methods of the invention.

A particular advantage of the technique of the invention is the statistical improbability of a particular combination of two sub-sequences occurring more than once in a given population

25 of DNA molecules. This is in contrast to the relatively high probability of random occurrences of a single sub-sequence in a given population of DNA molecules. Taking the example of a sub-sequence composed of 10 nucleotides, the total possible number of such sequences is approximately 1 million. This is a relatively small number in relation to the size of the human genome, for example, which contains three thousand million base-pairs.

30 Random occurrences of a particular 10 nucleotide sequence might therefore be expected to occur relatively frequently within the human genome. In contrast, the number of possible combinations of two different 10-nucleotide sequences is approximately five hundred million

million million. This number is much larger than the number of base-pairs in the human genome, and the chances of a particular combination of two 10-nucleotide sequences occurring more than once in the population of DNA molecules comprising the human genome are correspondingly reduced.

5   The technique of the invention may therefore be used to implement highly-specific DNA-based detection procedures for a particular genetic material with a low probability of obtaining spurious results due to the random occurrence of matching DNA sequences in the sample.

An example of the application of the process of the invention to discriminate between two

10   different DNA molecules is illustrated in Figure 18. The nucleotide sequences given in this diagram are for illustrative purposes only and do not represent the sequences of actual biological materials.

Referring to the diagram, if it is desired to discriminate between the two double-stranded 100-base-pair DNA sequences, sequence X and sequence Y, this may be achieved using a set

15   of oligonucleotide primers of appropriate sequence. The diagram illustrates a set of eight 15-base oligonucleotide primers A to H inclusive. The 8 x 8 matrix tables in the lower part of the diagram represent the results which would be obtained by application of the process of the invention using this set of primers and the two DNA sample molecules X and Y. In each table, the columns represent the oligonucleotides attached to the solid-support during the first

20   step of the process, in which sequence information from the sample molecules is transcribed into solid-supported primer-extension product. Conversely, the rows of each table represent the solution-phase oligonucleotides used during the second step of the process, during which labelled second-strands complementary to the support-bound polynucleotides are produced. The presence of a "+" symbol in each table indicates a combination which gives rise to

25   incorporation of labelled molecules into the product, whereas the presence of a "-" symbol denotes the absence of labelled molecules.

As indicated by the sections highlighted in bold type, sequence X contains two sequences of nucleotides which match those of two of the eight primers. A match for primer C is found in the "top" strand of sequence X, reading from left to right, while a match for primer B is

30   found in the "bottom" strand, in which the 5'-terminus is at the right-hand end, and the sequence therefore reads from right to left. Reference to the matrix table for sequence X shows that a positive result is obtained for this combination of primers, which may be

generated in two different ways, depending on which of the two primers is attached to the
solid support.

Similarly, sequence Y contains matching sequences for primer A in the "top" strand, reading
from left to right, and for primer H in the "bottom" strand, reading from right to left.

5   Reference to the matrix table for sequence Y again shows that a positive result is obtained
for both of the ways in which this combination of primers may be generated.

As will be apparent to those skilled in the art of electronic logic circuitry, the matrix tables
shown in the figure are analogous to the "truth tables" used in analysis of semiconductor

10   logic devices, and the process of the invention may be regarded as providing the functionality
of an "AND" logic gate for DNA sequences. As such, the outcome of a particular experiment
may be predicted in advance if the nucleotide sequences of the sample molecules and the
primers are known, allowing analytical procedures for specific applications to be designed
"in-silico", using computer modelling techniques.

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As will be appreciated by those skilled in the art of DNA analysis and combinatorial
chemistry, the operations involved in practising the methods of the invention may readily be
automated by using standard items of laboratory equipment designed for use in combinatorial
chemical synthesis and high-throughput screening. These may include but are not restricted

20   to thermal cyclers, automated pipettors, diluters and dispensers, robotic systems, two-
dimensional (X-Y) positioning systems and three-dimensional (X-Y-Z) motion systems,
multi-tube racks, multi-compartment reaction blocks and multi-well plate systems, plate
readers, autosampling apparatus, spectrophotometers and spectrofluorimeters including flow-
through spectrophotometers and spectrofluorimeters. In addition, equipment designed for

25   specific applications in oligonucleotide synthesis may also be adapted for the practice of the
invention. Such equipment may include but is not restricted to automated synthesisers such
as those described in my US patent 4,728,502 entitled "Apparatus for the chemical synthesis
of oligonucleotides" and in my GB patent 2,347,141 entitled "Combinatorial synthesiser".

30   The foregoing description illustrates embodiments of the invention. However, the invention
is not restricted to the specific embodiments described, and many variations of the invention
will be apparent to those skilled in the arts of biochemical analysis and DNA technology

without departing from the spirit of the invention. For example, a wide variety of enzymes and enzymatic processes could be used in the practice of the invention in place of the polymerase chain reaction, including reverse transcriptase in order to practise the technique of RT-PCR, or DNA ligase in order to practise the ligase chain reaction.

5

Similarly, although the subdivided support surfaces hereinbefore described are arranged in a rectangular format, other formats, for example formats employing a hexagonal or triangular layout of sub-zones, would be equally effective.

10   Accordingly, the scope of the invention should not be determined by reference to the foregoing description, but solely by reference to the appended claims along with their full scope of equivalents.

CLAIMS

1.   A process for transcription or amplification of nucleotide sequence information from genetic material by chain extension of oligonucleotide primers characterised in that during

5 the performance of the process:

(i) at least two oligonucleotides are in solution, and

(ii) at least two other oligonucleotides are attached to solid supports, and

(iii) the nucleotide-sequence of at least one solid-support-bound oligonucleotide is partially or completely identical with or complementary to the nucleotide-sequence of at least one

10 oligonucleotide in solution.

2.   A process for transcription or amplification of sequence information from a polynucleotide by chain extension of oligonucleotide primers characterised in that during the performance of the process:

15 (i) at least two oligonucleotide primers are in solution, and

(ii) at least one other oligonucleotide primer is attached to a solid support, and

(iii) the nucleotide-sequence of at least one solid-support-bound oligonucleotide is partially or completely identical with or complementary to part of the nucleotide-sequence of the polynucleotide.

20

3.   An apparatus for the analysis of analytes which comprises a multiplicity of solid . support surfaces, a multiplicity of oligonucleotides or polynucleotides attached to said solid support surfaces, such that each surface bears a single oligonucleotide or polynucleotide,

25 identification means allowing each of said solid-support surfaces to be distinguished from other solid support surfaces, containment means allowing said solid-support surfaces to be contacted with solutions of analytes, enzymes or reagents, and closure means to allow agitation, heating or cooling without leakage.

30 4.   An apparatus for the analysis of analytes which comprises a single solid support surface segregated into zones, a multiplicity of oligonucleotides or polynucleotides attached to said zones, such that each zone bears a single oligonucleotide or polynucleotide,

identification means allowing each of said zones to be distinguished from other zones, containment means allowing said solid-support surface to be contacted with solutions of analytes, enzymes or reagents, and closure means to allow agitation, heating or cooling without leakage.

5

5.   The apparatus of claims 3 or 4 further comprising containment means allowing any individual solid-support surface to be contacted with a specific analyte, enzyme or reagent without so contacting other solid-support surfaces, such that a different analyte, enzyme or reagent may be contacted with every individual solid-support surface.

10

6.   The apparatus of claims 3 to 5 in which each solid support-surface or zone of said surface bears multiple oligonucleotides or polynucleotides in combination, such that each said surface or zone bears a different combination of oligonucleotides or polynucleotides, said combination comprising a sub-set of the totality of oligonucleotides or polynucleotides

15   attached to all said surfaces or zones of the apparatus.

7.   An apparatus comprising a single solid support surface segregated into zones as defined in any of claims 4 to 6 further comprising division means allowing each of said zones to be physically separated from other zones, thereby enabling the entire support surface

20   to be physically divided into individual zones.

8.   A process for the preparation of multiple polynucleotides attached to a plurality of support surfaces which comprises the steps of:

(i) providing a plurality of support surfaces

25   (ii) covalently linking one or more oligonucleotides to each of said surfaces by means of a stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the support surface, such that each of said surfaces bears a different oligonucleotide or combination of oligonucleotides,

(iii) contacting each of said support surfaces with one or more additional oligonucleotides

30   and a nucleic acid or mixture of nucleic acids in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

(iv) optionally heating or altering the pH of said solid-supported oligonucleotide or

oligonucleotides, mixture of oligonucleotides and nucleic acids, enzyme or enzymes,

substrates and co-factors so as to effect dissociation of double-stranded molecules,

(v) optionally cooling or altering the pH of said solid-supported oligonucleotide or

5   oligonucleotides, mixture of oligonucleotides and nucleic acids, enzyme or enzymes,

substrates and co-factors so as to effect further transfer of genetic sequence data from the

nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface, and

(vi) optionally repeating steps (iv) to (v) one or more times in a cyclic manner, so as to effect

conversion of individual oligonucleotides of the support surface to polynucleotides

10   containing genetic sequence data derived from the nucleic acid or mixture of nucleic acids.

9.   A process for the preparation of multiple polynucleotides attached to a support

surface which comprises the steps of:

(i) providing a support surface

15   (ii) segregating the surface into discrete zones

(iii) covalently linking a plurality of oligonucleotides to said zones by means of a stable

linker moiety covalently linked to the 5'-terminus of each oligonucleotide and covalently

linked to the support surface, such that each individual

zone bears a different oligonucleotide or combination of oligonucleotides,

20   (iv) contacting said support surface with one or more additional oligonucleotides and a

nucleic acid or mixture of nucleic acids in the presence of an enzyme or enzymes together

with enzyme substrates and co-factors so as to transfer genetic sequence data from the

nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

(v) optionally heating or altering the pH of said solid-support-bound oligonucleotide or

25   oligonucleotides, mixture of oligonucleotides and nucleic acids, enzyme or enzymes,

substrates and co-factors so as to effect dissociation of double-stranded molecules,

(vi) optionally cooling or altering the pH of said solid-support-bound oligonucleotide or

oligonucleotides, mixture of oligonucleotides and nucleic acids, enzyme or enzymes,

substrates and co-factors so as to effect further transfer of genetic sequence data from the

30   nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface, and

(vii) optionally repeating steps (v) to (vi) one or more times in a cyclic manner, so as to effect conversion of individual oligonucleotides of the support surface to polynucleotides containing genetic sequence data derived from the nucleic acid or mixture of nucleic acids.

5   10.   A process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

(i) applying the process of claims 8 or 9 to produce a support surface or plurality of support surfaces bearing multiple polynucleotides containing genetic sequence data derived from the nucleic acid or mixture of nucleic acids,

10 (ii) contacting at least one of said support surfaces with one or more oligonucleotide or polynucleotide probes capable of hybridising to polynucleotides of the support surface or surfaces,

(iii) washing the support surface or surfaces to remove unhybridised oligonucleotide or polynucleotide probes,

15 (iv) measuring the quantity of oligonucleotide or polynucleotide probe hybridised to each polynucleotide of the support surface or surfaces, and

(v) optionally washing the support surface or surfaces under conditions sufficient to remove hybridised oligonucleotide or polynucleotide probes and repeating steps (ii) to (iv) one or more times using a different oligonucleotide or polynucleotide probe or probes in each

20 iteration.

11.   A process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

(i) applying the process of claims 8 or 9 to produce a support surface or plurality of support

25 surfaces bearing multiple polynucleotides containing genetic sequence data derived from the nucleic acid or mixture of nucleic acids,

(ii) contacting at least one of said support surfaces with one or more oligonucleotide primers in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to produce one or more polynucleotides complementary to the polynucleotides of the

30 support surface or surfaces,

(iii) optionally heating or altering the pH of said support surface or surfaces, oligonucleotide primer or primers, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(iv) optionally cooling or altering the pH of said support surface or surfaces, oligonucleotide

5   primer or primers, enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides of the support surface or surfaces,

(v) optionally repeating steps (iii) to (iv) one or more times in a cyclic manner, so as to effect conversion of the primer oligonucleotide or oligonucleotides to polynucleotides complementary to the polynucleotides of the support surface or surfaces,

10   (vi) hybridising said complementary polynucleotides to the polynucleotides of the support surface or surfaces,

(vii) washing the support surface or surfaces to remove unhybridised polynucleotides,

(viii) measuring the quantity of complementary polynucleotide hybridised to each polynucleotide of the support surface or surfaces, and

15   (ix) optionally washing the support surface or surfaces under conditions sufficient to remove hybridised complementary polynucleotides and repeating steps (ii) to (viii) one or more times using a different oligonucleotide primer or primers in each iteration.

12.   A process for the analysis of a nucleic acid or mixture of nucleic acids which

20   comprises the steps of:

(i) providing a multiplicity of support surfaces each equipped with unique identification means,

(ii) covalently linking each of a set of n oligonucleotides, where n is an integral number greater than unity, to one of said support surfaces by means of a stable linker moiety

25   covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the support surface, such that each individual oligonucleotide is attached to an individual support surface,

(iii) contacting all of said support-surfaces with a solution containing a mixture of all n oligonucleotides and the nucleic acid or mixture of nucleic acids to be analysed in the

30   presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides bound to the support surfaces,

(iv) optionally heating or altering the pH of said support surfaces, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(v) optionally cooling or altering the pH of said support surfaces, mixture of oligonucleotides

5   and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

(vi) optionally repeating steps (iv) and (v) one or more times in a cyclic manner, so as to produce n sets of support-bound polynucleotides by effecting conversion of individual

10   support-bound oligonucleotides to polynucleotides containing genetic sequence data derived from the mixtures of nucleic acids,

(vii) washing said support surfaces under denaturing conditions to denature double-stranded molecules and remove oligonucleotides or polynucleotides hybridised to said support-bound polynucleotides,

15   (viii) contacting one or more of said n support surfaces with a solution containing one of said n oligonucleotide primers in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to produce polynucleotides complementary to the polynucleotides bound to said support surfaces,

(ix) optionally heating or altering the pH of said support surfaces, oligonucleotide primers,

20   enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(x) optionally cooling or altering the pH of said support surfaces, oligonucleotide primers, enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides bound to said support surfaces,

25   (xi) optionally repeating steps (ix) to (x) one or more times in a cyclic manner, so as to effect conversion of the primer oligonucleotide to polynucleotides complementary to the polynucleotides bound to said support surfaces,

(xii) hybridising said complementary polynucleotides to the polynucleotides bound to said support surfaces,

30   (xiii) washing said support surfaces under non-denaturing conditions to remove unhybridised polynucleotides,

(xiv) separating each of said support surfaces from other support surfaces,

(xv) washing each of said support surfaces under denaturing conditions to denature double-stranded molecules and release hybridised polynucleotides into solution,

(xvi) analysing the hybridised polynucleotides released into solution from each said support surface, and

5   (xvii) optionally repeating steps (viii) to (xvi) one or more times using a different member of the said set of n oligonucleotide primers in each iteration.

13.   A process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

10   (i) providing a multiplicity of support surfaces each equipped with unique identification means,

(ii) covalently linking each of a set of n oligonucleotides, where n is an integral number greater than unity, to one of said support surfaces by means of a stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the

15   support surface, such that each individual oligonucleotide is attached to an individual support surface,

(iii) contacting all of said support-surfaces with a solution containing a mixture of all n oligonucleotides and the nucleic acid or mixture of nucleic acids to be analysed in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to

20   transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides bound to the support surfaces,

(iv) optionally heating or altering the pH of said support surfaces, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

25   (v) optionally cooling or altering the pH of said support surfaces, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

(vi) optionally repeating steps (iv) and (v) one or more times in a cyclic manner, so as to

30   produce n sets of support-bound polynucleotides by effecting conversion of individual support-bound oligonucleotides to polynucleotides containing genetic sequence data derived from the mixtures of nucleic acids,

(vii) washing said support surfaces under denaturing conditions to denature double-stranded molecules and remove oligonucleotides or polynucleotides hybridised to said support-bound polynucleotides,

(viii) individually contacting each of said n support surfaces with a solution containing one of said n oligonucleotide primers in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to produce polynucleotides complementary to the polynucleotides bound to said support surface, such that every one of said n support surfaces is contacted with a unique member of the set of n oligonucleotide primers,

(ix) optionally heating or altering the pH of every said support surface, oligonucleotide primer, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(x) optionally cooling or altering the pH of every said support surface, oligonucleotide primer, enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides bound to said support surface,

(xi) optionally repeating steps (ix) to (x) one or more times in a cyclic manner, so as to effect conversion of the primer oligonucleotide to polynucleotides complementary to the polynucleotides bound to said support surface,

(xii) hybridising said complementary polynucleotides to the polynucleotides bound to said support surface,

(xiii) washing said support surface under non-denaturing conditions to remove unhybridised polynucleotides,

(xiv) washing said support surface under denaturing conditions to denature double-stranded molecules and release hybridised polynucleotides into solution,

(xv) analysing the hybridised polynucleotides released into solution from each said support surface, and

(xvi) optionally repeating steps (viii) to (xv) one or more times using for each of said n support surfaces a different member of the said set of n oligonucleotide primers in each iteration.

14.   A process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

(i) providing a support surface,

(ii) segregating the surface into n discrete zones, where n is an integral number greater than unity,

(iii) providing each of said zones with unique identification means,

(iv) covalently linking each of a set of n oligonucleotides to one of said zones by means of a

5 stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the support surface, such that each individual oligonucleotide is attached to an individual zone of the support surface,

(v) contacting said support-surface with a solution containing a mixture of all n oligonucleotides and the nucleic acid or mixture of nucleic acids to be analysed in the

10 presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides attached to the support surface,

(vi) optionally heating or altering the pH of said support surface, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation

15 of double-stranded molecules,

(vii) optionally cooling or altering the pH of said support surface, mixture of oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic acids to the oligonucleotides of the support surface,

20 (viii) optionally repeating steps (vi) and (vii) one or more times in a cyclic manner, so as to produce n sets of support-bound polynucleotides by effecting conversion of individual support-bound oligonucleotides to polynucleotides containing genetic sequence data derived from the mixtures of nucleic acids,

(ix) washing said support zones under denaturing conditions to denature double-stranded

25 molecules and remove oligonucleotides or polynucleotides hybridised to said support-bound polynucleotides,

(x) contacting one or more of said n support zones with a solution containing one of said n oligonucleotide primers in the presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to produce polynucleotides complementary to the

30 polynucleotides bound to said support zones,

(xi) optionally heating or altering the pH of said support zones, oligonucleotide primer, enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded molecules,

(xii) optionally cooling or altering the pH of said support zones, oligonucleotide primer,

enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides bound to said support zones,

(xiii) optionally repeating steps (xi) to (xii) one or more times in a cyclic manner, so as to effect conversion of the primer oligonucleotide to polynucleotides complementary to the polynucleotides bound to said support zones,

(xiv) hybridising said complementary polynucleotides to the polynucleotides bound to said support zones,

(xv) washing said support zones under non-denaturing conditions to remove unhybridised polynucleotides,

(xvi) separating each of said support zones from other support zones,

(xvii) washing each said support zone under denaturing conditions to denature double-stranded molecules and release hybridised polynucleotides into solution,

(xviii) analysing the hybridised polynucleotides released into solution from each said support zone, and

(xix) optionally repeating steps (x) to (xviii) one or more times using in each iteration a solution of a different oligonucleotide primer selected from the set of n oligonucleotide primers.

15.   A process for the analysis of a nucleic acid or mixture of nucleic acids which comprises the steps of:

(i) providing a support surface,

(ii) segregating the surface into n discrete zones, where n is an integral number greater than unity,

(iii) providing each of said zones with unique identification means,

(iv) covalently linking each of a set of n oligonucleotides to one of said zones by means of a stable linker moiety covalently linked to the 5'-terminus of each oligonucleotide and covalently linked to the support surface, such that each individual oligonucleotide is attached to an individual zone of the support surface,

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(v) contacting said support-surface with a solution containing a mixture of all n

oligonucleotides and the nucleic acid or mixture of nucleic acids to be analysed in the

presence of an enzyme or enzymes together with enzyme substrates and co-factors so as to

transfer genetic sequence data from the nucleic acid or mixture of nucleic acids to the

5   oligonucleotides attached to the support surface,

(vi) optionally heating or altering the pH of said support surface, mixture of oligonucleotides

and nucleic acids, enzyme or enzymes, substrates and co-factors so as to effect dissociation

of double-stranded molecules,

(vii) optionally cooling or altering the pH of said support surface, mixture of

10   oligonucleotides and nucleic acids, enzyme or enzymes, substrates and co-factors so as to

effect further transfer of genetic sequence data from the nucleic acid or mixture of nucleic

acids to the oligonucleotides of the support surface,

(viii) optionally repeating steps (vi) and (vii) one or more times in a cyclic manner, so as to

produce n sets of support-bound polynucleotides by effecting conversion of individual

15   support-bound oligonucleotides to polynucleotides containing genetic sequence data derived

from the mixtures of nucleic acids,

(ix) washing said support surface under denaturing conditions to denature double-stranded

molecules and remove oligonucleotides or polynucleotides hybridised to said support-bound

polynucleotides,

20   (x) physically dividing the support surface to form n individually-identifiable support zones

each bearing a set of support-bound polynucleotides derived by extension of one of the n

support-bound oligonucleotide primers,

(xi) individually contacting every one of said n support zones with a solution containing one

of said n oligonucleotide primers in the presence of an enzyme or enzymes together with

25   enzyme substrates and co-factors so as to produce polynucleotides complementary to the

polynucleotides bound to said support zone, such that every one of said n support zones is

contacted with a unique member of the set of n oligonucleotide primers,

(xii) optionally heating or altering the pH of every said support zone, oligonucleotide primer,

enzyme or enzymes, substrates and co-factors so as to effect dissociation of double-stranded

30   molecules,

(xiii) optionally cooling or altering the pH of every said support zone, oligonucleotide primer, enzyme or enzymes, substrates and co-factors so as to produce further polynucleotides complementary to the polynucleotides bound to said support zone,

(xiv) optionally repeating steps (xii) to (xiii) one or more times in a cyclic manner, so as to effect conversion of the primer oligonucleotide to polynucleotides complementary to the polynucleotides bound to said support zone,

(xv) hybridising said complementary polynucleotides to the polynucleotides bound to said support zone,

(xvi) washing said support zone under non-denaturing conditions to remove unhybridised polynucleotides,

(xvii) washing said support zone under denaturing conditions to denature double-stranded molecules and release hybridised polynucleotides into solution,

(xviii) analysing the hybridised polynucleotides released into solution from each said support zone, and

(xix) optionally repeating steps (xi) to (xviii) one or more times using for each of said n support zones a different member of the said set of n oligonucleotide primers in each iteration.

16.   A process for the analysis of two or more distinct mixtures of nucleic acids which comprises the steps of:

(i) applying the process of claim 11 to produce a set of support surfaces or zones bearing a first set of immobilised polynucleotides containing genetic sequence data derived from a first mixture of nucleic acids, together with a first set of polynucleotides complementary to said first set of immobilised polynucleotides,

(ii) applying the process of claim 11 to produce a set of support surfaces or zones bearing a second set of immobilised polynucleotides containing genetic sequence data derived from a second mixture of nucleic acids, together with a second set of polynucleotides complementary to said second set of immobilised polynucleotides,

(iii) hybridising said first set of complementary polynucleotides to said first set of immobilised polynucleotides,

(iv) individually releasing and analysing the complementary polynucleotides hybridised to each of said first set of immobilised polynucleotides,

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(v) hybridising said second set of complementary polynucleotides to said second set of immobilised polynucleotides

(vi) individually releasing and analysing the complementary polynucleotides hybridised to each of said second set of immobilised polynucleotides,

5   (vii) optionally hybridising one or more members of said first set of complementary polynucleotides to one or more members of said second set of immobilised polynucleotides, individually releasing and analysing the complementary polynucleotides hybridised to each of said second set of immobilised polynucleotides,

(viii) optionally hybridising one or more members of said second set of complementary

10   polynucleotides to one or more members of said first set of immobilised polynucleotides, individually releasing and analysing the complementary polynucleotides hybridised to each of said first set of immobilised polynucleotides, and

(ix) optionally repeating steps (ii) and (v) to (viii) for further mixtures of nucleic acids.

15   17.   The process of any preceding claim in which a single support surface segregated into zones is physically divided into discrete zones after completing at least one step of the process and each such zone is processed separately during subsequent steps of the process.

18.   The process of any preceding claim in which the stable linker moiety is covalently

20   linked to the 5'-terminus of each oligonucleotide by means of covalent bonding to one or more atoms of the carbohydrate moiety of the 5'-terminal nucleotide.

19.   The process of any preceding claim in which the stable linker moiety is covalently linked to the 5'-terminus of each oligonucleotide by means of covalent bonding to one or

25   more atoms of the heterocyclic moiety of the 5'-terminal nucleotide.

20.   The process of any preceding claim in which the stable linker moiety comprises a covalently-bound functionality with a chemical equivalence of between 100 and 5000 picomoles per square millimetre of support surface.

30

21.   The process of any preceding claim in which the stable linker moiety is thermally stable in aqueous solution at 95 deg. C.

22.   The process of any preceding claim in which the stable linker moiety is hydrolytically stable in aqueous solution between pH 4 and pH 11.

5   23.   The process of any preceding claim in which the oligonucleotides contain internucleotide linkages selected from the group comprising phosphodiesters, phosphorothioates and methyl phosphonates.

24.   The process of any preceding claim in which one or more oligonucleotides or

10   polynucleotides contain labelled molecules selected from the group comprising fluorescent molecules, luminescent molecules, biotin-labelled molecules, radiolabelled molecules and enzyme conjugates.

25.   The process of any preceding claim in which the nucleic acid or mixture of nucleic

15   acids to be analysed is selected from the group comprising single-stranded DNA molecules, double-stranded DNA molecules and messenger RNA molecules.

26.   The process of any preceding claim in which the enzyme or enzymes are selected from the group comprising DNA polymerases, thermostable DNA polymerases, reverse

20   transcriptases, thermostable reverse transcriptases, DNA ligases and thermostable DNA ligases.

27.   The process of any preceding claim in which the enzyme substrates and co-factors are selected from the group comprising nucleoside triphosphates, fluorescent nucleoside

25   triphosphates, luminescent nucleoside triphosphates, biotin-labelled nucleoside triphosphates, amino-allyl nucleoside triphosphates, radiolabelled nucleoside triphosphates, metal salts, buffering agents, chelating agents, chaotropic agents and nuclease inhibitors.

28.   The apparatus of any preceding claim in which the support surface, containment

30   means and closure means comprise separate components adapted to be assembled to form the apparatus.

29.   The apparatus of any preceding claim in which each set of immobilised molecules further comprises control substances or indicia adapted for identification, alignment or calibration purposes.

30.   The apparatus and processes of any preceding claims further comprising automated handling equipment allowing high-throughput analysis of DNA samples under computer control.

31.   A computer program product directly loadable into the internal memory of a digital computer, comprising software code portions for controlling apparatus adapted to perform the process of the invention as defined in any preceding claim.

32.   A computer program product directly loadable into the internal memory of a digital computer, comprising software code portions for predicting the outcome of the process of the invention as defined in any preceding claim for any given combination of oligonucleotides in relation to the presence or absence in an analyte of one or more polynucleotides.
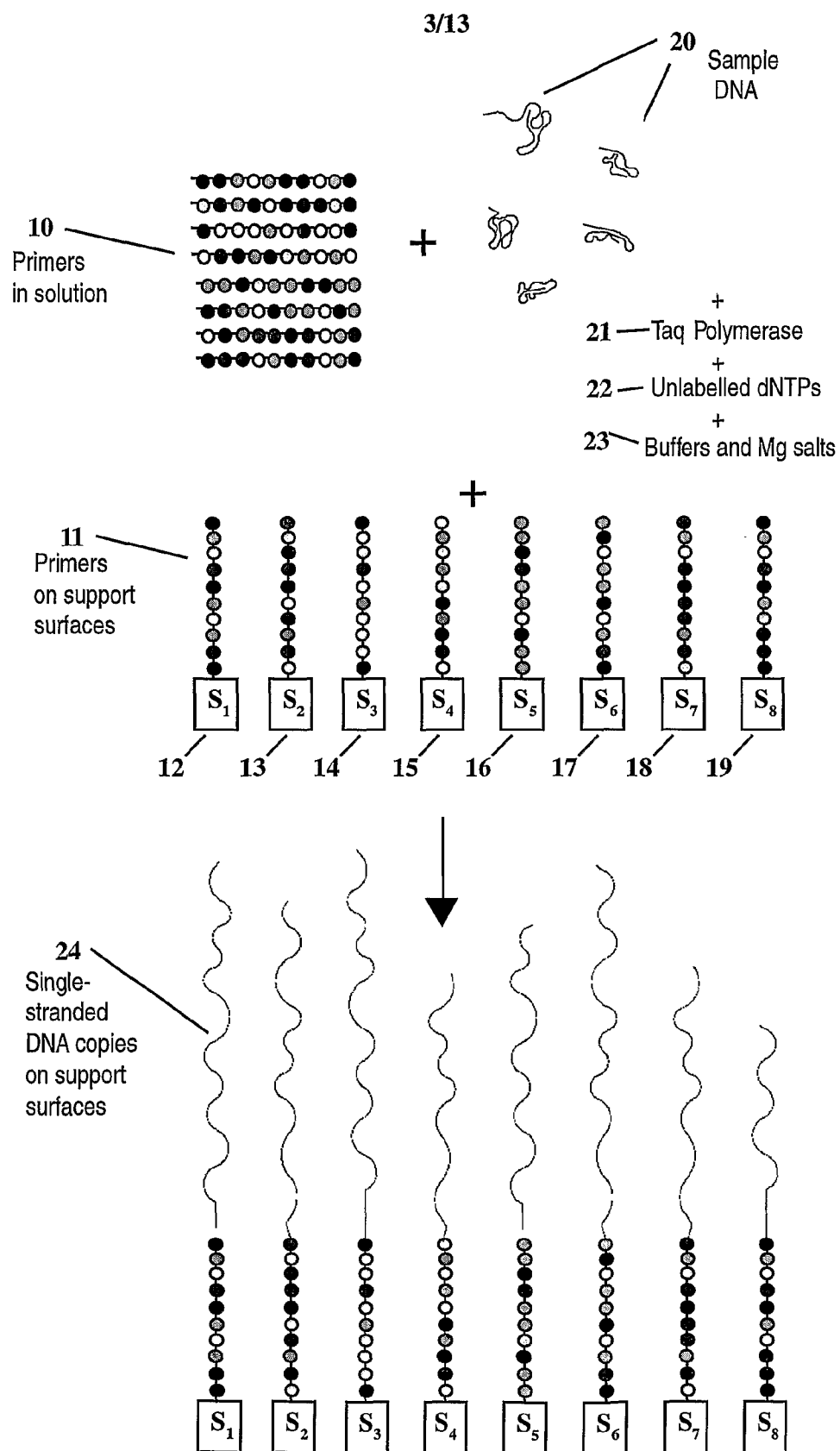
Figure 1

Figure 2

Figure 3

Figure 4

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25 Single primer

26

+ Polymerase +
Buffers, Mg salts
+
Labelled dNTPs

27

24
Single-
stranded
DNA copies
on support
surfaces

28

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

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Figure 11

Figure 12

Figure 13

Figure 14

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Figure 15

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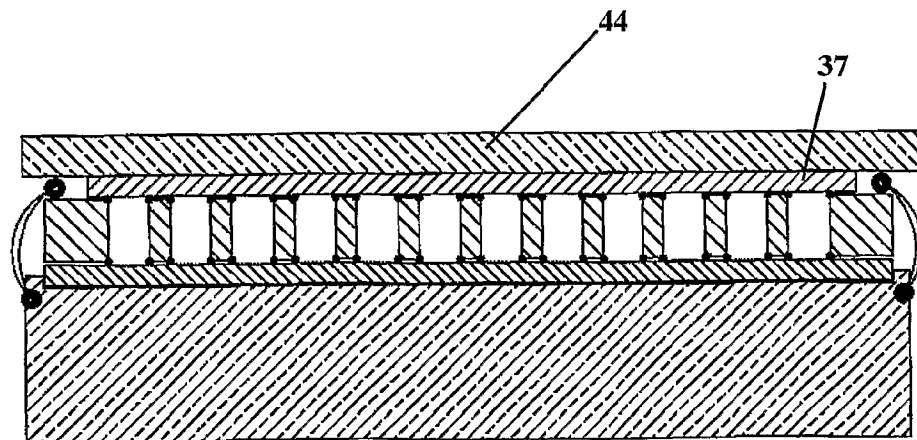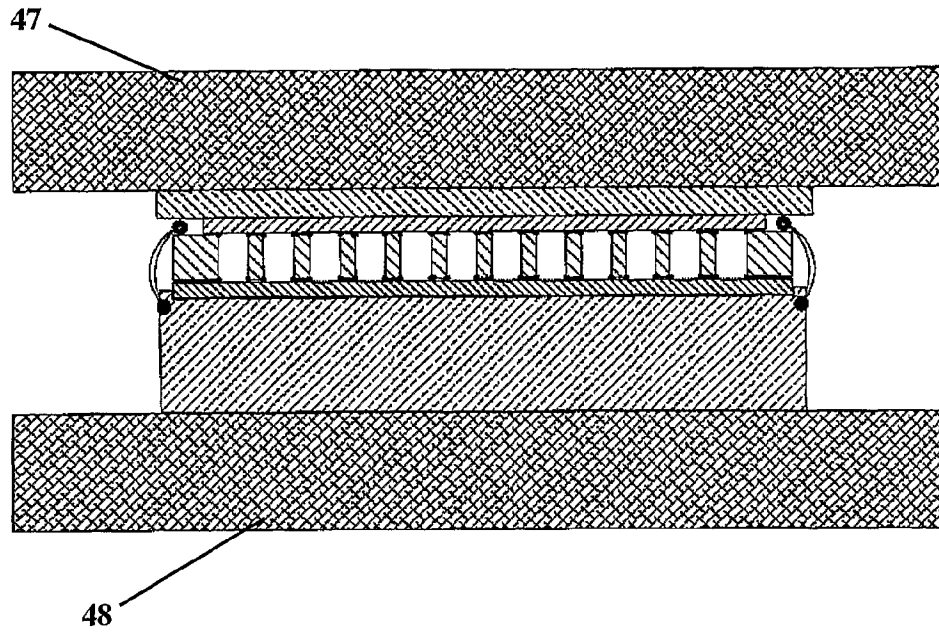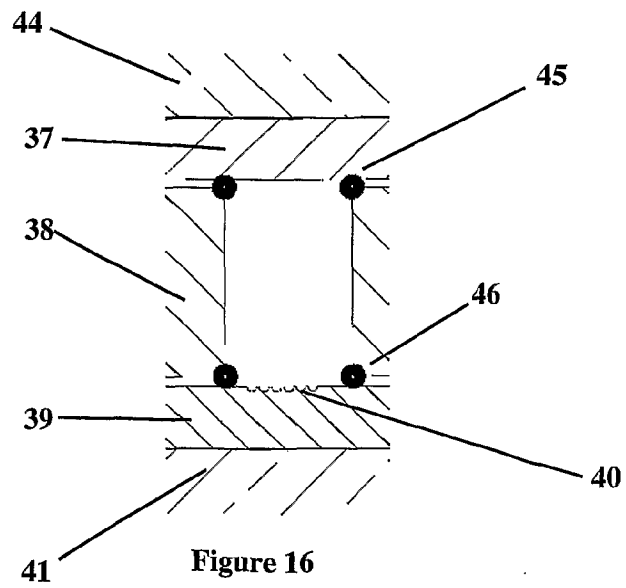
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Figure 16

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GTTTCGCTCC    TGCTCTGCCC    GTAGACCCGT    TTTGCCCGGA
TGATCCCTGG    GGTGACGCAG    CCTTGACGCA    AGGGAACGAG
CATCCCCCTG    GTCCACACGG    TTCCCCCGCT    CCACAGCAGT
GGACTGGAGT    TGGGGGACTC    TCCGCTCTGG    ACCCCCGAAG
TGCGCCCTTC    CTGCTGGGAC    GGAGGGTGTT    GGACCCTTAC

CCCAAGGTCC    AAGACCCCTC    GAGTCTCAGG    GGTGACTGTG
GGTGCGGGAA    AGATGCAGCC    TTATCGCCCC    CTACTGCCGT
CCAGATGCAC    TCACCACGGT    CCCGATTCGG    GGACTGCAGA
GTGACATGCC    CTTCACCCGA    TGCGGCTGAG    ACGGCGTATG
TCAGGGAGGT    CACCAGGTGA    ACGCACAACC    AACGGTGACC

CCCGGCATAA    TCGTTCCGCA    ACTCCTGCGA    CTGCTTAGGG
CCCGTTGGGA    CCTCTCGACA    GTCCCGTGGT    ACGCCAGTTC
TCTCCGCTTG    CATACCGTGG    CCACACTACC    TGGTCGCAGA
CCGAACACGG    TGAGCCTCAC    CACCCGGATG    GGACACCACT
CTCCATGGGG    AAGCCCGAGG    TGTAGCAGGG    AAGCGGCCTC

GGCACGTAAG    CCACGGGAAG    GACAGGAGGT    TCGGCGGTTC
ACGTAGCGTC    CAGCACTGAC    CAGTGCTGTG    GGCTTATGCC
CTGTTGCTAC    CCTCCAGTGT    GTCAGAGTCC    CTCGCTATCC
AAGTCCGCTC    TCCCACGCAA    AGCATGGCTC    GGTGCACGTT
CCCAGTCACT    TCAGAGCGCC    TGGCGTCCTT    ACACACGCTG

GGGCCACTCA    CAAGGGCAGA    TTCCCCGCGA    GGTGAACGCT
GGAGAGACTC    GGCAGGCTGT    GGGTGTGTAG    CCAACGTCGT
TCCACTCCTG    AACGGCGACA    AGGATGCCAG    GATGCCAGAC
CACAGAGGGA    CACCCCTGAG    AATGCCGCAG    GTCCGTATGG
GGGTTTGGCA    CCTTCGGAAG    GGATGCCACT    GACCAATGCC

GTGGCATCTC    AAGGCTCACC    AGACGATGGG    GGGCCAATGT
CATCGCCGCA    AGAGCCGTCA    AAGCCTGCGA    GACGTGGTGA
ACAGCCTGCT    AGGCAGAGCA    GGGTCTCGGT    GTGGAGTCAG
GGCTGCAATC    AGCAGCGCAC    GGTCGATCTG    TGAGGGTCCC
GGCTGCGACA    CAAACGTGGG    AGTCGCCCTT    AGCCGTGGAA

CAAAGGGCGG    AAGTGCACGG    CAATCGGGTC    AACGGGCGTC
CTGAACCGCT    CCCTACTGGT    AAGAGGGCGT    GGCAAACCCT
TCTCGCCTAC    GGCAGGCAAG    GGTTCCTCTG    ACGAGAGGCA
GTAGGCCTCA    TCGCTTCTCC    GAACGAGGGT    CTTGGCACGA
ACCGCATGGG    AAGAGGCCAG    TTTGCCCCGT    TCTTCGGAGG

GGCATCGGCT    TGCCGCACTT    ACGGCGATGA    AAGGCACGAG
AGCCGTTCAG    ACGAGCATGG    GACTCTAACC    CCTCACGTCC
GGGTCCAAAG    AAGCCCCCCA    ACGCTGCGAC    TCGCGGAACC
CTATCCTGCC    TCGCTGGTGT    TGGTGCACTC    GGCAAAGCTG
GTCGTAGCGG    TCGGGCATCA    GACACAGCCC    CCTGTTCCCT

ACTCCACGTC    GGGAACCCGT    GTCCATGCAG    GTGTCGAGTC
CACCGCAGTT    TCGCTGCGGA    AACGGCGGTC    TCAGCACAGG
AGCCAGGCTG    AAGGCTGCTG    CTTCCAGGAC    TGTCCTGCGT
GGCGTAAGTC    GGGGGAGATG    AGCCGGGTAA    ACCACGCCTT
GGGTGCAGTT    CTGTGTGCTC    TGATGCCGCT    GAGTCCTCAC

CACACCGTGT    GGCGCGTTAG    ACCGTGCCGT    AACCCTTCCC
GTCCTCGTGT    GACGAGCAGG    TGACCAGGCA    AGTTCCGCGA
ACGGTTCCAC    GGCTGCCAGT    CACGGACCGA    GTTGCGCAGT
GTCTTGGGCA    TGGAGTCCCC    TCGCAGCGTT    TGACAGCCCC
GTCACCTGCT    CCCGTCTACC    CTGCAATGGG    TCTGCCTGGA
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Figure 17

SEQUENCE X =

```
(5'-) AGTTCTTCCGTAGCTGAGTTCGATTCGATTCGATCGAGAGCTACGTTCGATCGAGATACGCTAGCTCATACTGGCCCTAGCTTACTAACTTAGGATTAGTAGCTC
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
(3'-) TCAAGAAGGCATCGACTCGACTAAGCTAAGCTAGCTCGATCGAGTATGCGATGCAAGCTAGCTATGCGATCGAGTATGACCGGGATCGAATCGAATGATTGAATCCTAATCATCGAG
```

SEQUENCE Y =

```
(5'-) TGCCATACGAAGTTCAGCAGCATCGCTATCGTGTCTAGCATCATAGCTCTACGACTACAGAGACTTTAGCTACGTACGACTGATGCATCCGACTAGCTCTAGCTA
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
(3'-) ACGGTATGCTTCAAGTCGTAGCGATAGCACAGATCGTAGTATCGAGAGATGCTGAGTGTCTGAAATCGATGCATGCTGACTACGTAGGCTGATCGAGATCGAT
```

Primer sequences
(all 5'->3')

PRIMER A = ACGAAGTTCAGCATC　　　PRIMER B = TAAGCTAGGGCCAGT
PRIMER C = GTAGCTGATTCGATT　　　PRIMER D = ATGATCCATGTTACT
PRIMER E = ATTCTGGTATGCTAG　　　PRIMER F = GATCTTTAGCTAGTC
PRIMER G = ATAATCCGTAGCTAT　　　PRIMER H = AGTCGTACGTAGCTA

SEQUENCE X RESULTS:

<< solid-support-bound primers >>

	A_1	B_1	C_1	D_1	E_1	F_1	G_1	H_1
A_2	-	-	-	-	-	-	-	-
B_2	-	-	+	-	-	-	-	-
C_2	-	+	-	-	-	-	-	-
D_2	-	-	-	-	-	-	-	-
E_2	-	-	-	-	-	-	-	-
F_2	-	-	-	-	-	-	-	-
G_2	-	-	-	-	-	-	-	-
H_2	-	-	-	-	-	-	-	-

primers for labelled strand

SEQUENCE Y RESULTS:

	A_1	B_1	C_1	D_1	E_1	F_1	G_1	H_1
A_2	-	-	-	-	-	-	-	+
B_2	-	-	-	-	-	-	-	-
C_2	-	-	-	-	-	-	-	-
D_2	-	-	-	-	-	-	-	-
E_2	-	-	-	-	-	-	-	-
F_2	-	-	-	-	-	-	-	-
G_2	-	-	-	-	-	-	-	-
H_2	+	-	-	-	-	-	-	-

primers for labelled strand

Figure 18